Program/Abstract # 253
GABAergic differentiation in the basal ganglia requires retinoic acid signaling
Christina Chatzi[a], Thomas Brade[b], Gregg Duester[b]
[a]*Sanford-Burnham Med Research Institute, Development and Aging, La Jolla, CA, USA*
[b]*Sanford Burnham, La Jolla, CA, USA*

Although retinoic acid (RA) has been implicated as an extrinsic signal regulating forebrain neurogenesis, the processes regulated by RA signaling remain unclear. Here, analysis of retinaldehyde dehydrogenase mutant mouse embryos lacking RA synthesis demonstrates that RA generated by Raldh3 in the subventricular zone of the basal ganglia is required for GABAergic differentiation, whereas RA generated by Raldh2 in the meninges is unnecessary for development of the adjacent cortex. Neurospheres generated from the lateral ganglionic eminence (LGE), where Raldh3 is highly expressed, produce endogenous RA which is required for differentiation to GABAergic neurons. In Raldh3−/− embryos, LGE progenitors fail to differentiate into either GABAergic striatal projection neurons or GABAergic interneurons migrating to the olfactory bulb and cortex. We describe conditions for RA treatment of human embryonic stem cells that result in efficient differentiation to a heterogeneous population of GABAergic interneurons but not GABAergic striatal projection neurons, thus providing an in vitro method for generation of GABAergic interneurons. Our observation that endogenous RA is required for generation of LGE-derived GABAergic neurons in the basal ganglia establishes a key role for RA signaling in development of the forebrain.

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Program/Abstract # 254
MicroRNAs in dopamine progenitor specification
Angela Anderegg[a], Hsin Pin Lin[b], Beth Yun[a], Brian Harfe[c], Randy Johnson[d], Raj B. Awatramani[e]
[a]*Chicago, IL, USA*
[b]*Northwestern, Chicago, USA*
[c]*University of Florida, Gainesville, FL, USA*
[d]*University of Texas, Houston, TX, USA*
[e]*Northwestern Univ. Neurology, Chicago, IL, USA*

One hallmark of Parkinson's disease (PD) is the devastating motor symptoms that present when midbrain dopamine neuron (DA) numbers fall below a critical threshold. Accordingly, the original number of DAs an individual produces during fetal development could significantly contribute to disease susceptibility. That is, smaller DA pools may increase the risk for acquiring PD, while larger DA populations may enhance one's ability to withstand genetic and environmental insults that result in disease symptoms. Thus, a comprehensive understanding of the molecular programs that directs neural progenitors toward a DA fate will critically impact our knowledge about PD susceptibility as well as aid in the generation of PD therapeutics, such as programming embryonic stem cells into dopamine producing cells for transplantation. MicroRNAs are ~22 bp RNAs that serve to diminish mRNA stability and/or translation of cognate target genes, and are critical facilitators of key developmental events. We have identified a microRNA that is expressed in specific regions within the embryonic midbrain, including DA progenitors. We have used a conditional mouse genetics approach to evaluate the function of this microRNA, and reveal the upstream and downstream elements of this microRNA. Our results point to a model wherein this microRNA serves to spatially restrict the number of DA progenitors. This study sheds light on a novel function of microRNAs in cell fate specification events in the embryonic midbrain, and thus may be informative in understanding mechanisms that control DA numbers.

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Program/Abstract # 255
A genetic modifier screen of midline to identify candidate enhancer and suppressor genes that regulate interommatidial bristle formation in the adult drosophila eye
Deepak Kumar, Sandra Leal
University of Southern Mississippi, Hattiesburg, MS, USA

The Drosophila T-box transcription factor midline (mid) regulates cell-fate specification in multiple tissues across diverse invertebrate and vertebrate species. However, to date, the complex mechanisms by which mid regulates cell-fate specification are not yet completely understood. Developmental expression profile studies between Mid proteins and transcription factors known to specify motor neuron and interneuron fates within the central nervous system (CNS) reveal little co-expression between these factors (Leal et al., 2009). Thus, to further our understanding of mid function as a cell-fate determinant, we are using a genetic modifier screen based on RNA interference (RNAi) methodology to identify genes that suppress or enhance a dosage-sensitive RNAi interommatidial bristle mutant phenotype observed when mid transcripts are reduced in the eye imaginal disks of third-instar larvae heterozygous mutant for third chromosomal deficiencies. A follow-up rescreening assay will determine whether identified gene candidates modify a CNS-specific and dosage-sensitive mid mutant phenotype that affects even-skipped (eve) expression in a subset of neurons. Presently, we have identified several third chromosomal regions harboring enhancer or suppressor candidate genes that regulate interommatidial bristle formation. We are currently assaying mutant alleles of these prospective enhancer/suppressor gene candidates to identify bona fide mid-interacting genes. The goal of this research is to advance our understanding of mid function as an integral regulator of conserved cell-fate specification pathways within the CNS and other tissues.

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Program/Abstract # 256
The polycomb repressive complex PRC2 regulates retinal differentiation in *Xenopus*
Issam Aldiri[a], Gert Jan C. Veenstra[b], Monica Vetter[c]
[a]*University of Utah Neurobiology & Anatomy, Salt Lake City, UT, USA*
[b]*Radboud University Nijmegen, Nijmegen, Netherlands*
[c]*University of Utah, Salt Lake City, UT, USA*

The mechanisms that govern the transition of retinal progenitors from proliferation to differentiation are not fully understood. Studies have established that the histone methyl transferase complex PRC2, which can trimethylate lysine 27 on histone H3 (H3K27me3), is required for the proper progression from proliferation to differentiation in a variety of biological contexts. Here we report the involvement of PRC2 in regulating the transition from retinal proliferation to differentiation during eye development. We show that the transcripts of the core subunits of PRC2 are coincidently expressed in retinal progenitors and are downregulated upon retinal differentiation. Surprisingly, we found that the levels of H3K27me3 and the expression of the PRC2 binding partner Jarid2 greatly increase in terminally differentiated cells. Inhibition of Xez, the catalytic subunit of PRC2 using a translation blocking morpholino leads to a marked decrease in H3K27me3 in retinal cell types. Blocking Xez causes a reduction in eye size and inhibition of

differentiation genes. Importantly, targeted knockdown of Xez in retinal progenitors biases cell fate toward late born cell types, suggesting that retinal differentiation is delayed or inhibited. ChIP-seq analysis shows that H3K27me3 specifically decorates a subset of genes expressed in the eye, some of which are known negative regulators of retinal differentiation. Our data establishes PRC2 as a major player in retinal neurogenesis and suggests that it may have multiple roles in eye development, including regulation of retinal proliferation and/or differentiation. This work was supported by NIH grant# EY012274 to MLV.

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Program/Abstract # 257
The proneural target gene Sbt1 regulates neurogenesis in the Xenopus retina

Kathryn B. Moore[a], Mary Logan[b], Issam Al Diri[c], Derek Bunch[c], Monica Vetter[d]
[a]*University of Utah, Dept of Neurobiology & Anatomy, Salt Lake City, UT, USA*
[b]*Jungers Center for Neurosciences Research, Department of Neurology, Porland, OR, USA*
[c]*University of Utah, Salt Lake City, UT, USA*
[d]*Salt Lake City, UT, USA*

Proneural transcription factors are key regulators of retinal neurogenesis, activating a genetic cascade that executes a neuronal differentiation program in progenitors. Our study focuses on understanding this differentiation program in retinal progenitors by examining bHLH target gene function. The proneural target gene sbt1 encodes a novel protein with no conserved functional motifs. We determined the spatial and temporal expression of sbt1 and sought to gain insight into its function during retinal development. Our analysis showed that sbt1 is transiently expressed in late proliferating/early differentiating cells in the Xenopus retina and is localized both at the membrane and in the nucleus. Overexpression of sbt1 in progenitors promoted differentiation of early born retinal neurons, and enhanced the ability of the bHLH factor Ath5 to promote neurogenesis. Conversely, inhibition of SBT1 translation in retinal progenitors prevented or delayed retinal neuron differentiation, resulting in an increase in Müller glia/progenitors. sbt1 loss of function in progenitors blocked the expression of differentiated retinal neuron markers, suggesting that it is required for full proneural function. In addition, sbt 1overexpression caused a reduction in mitotic cells as measured by phospho-histone H3 staining. We performed a yeast 2-hybrid screen for SBT1 interactors and have isolated several potential protein partners, including proteins involved in cell cycle regulation. We propose that sbt1 is expressed in retinal progenitors as they initiate neuronal differentiation, and that it functions downstream of proneural bHLH factors during retinal development, perhaps by regulating cell cycle exit. Supported by NEI EY012274 (MLV).

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Program/Abstract # 258
Loss of Llgl1 results in neuroepithelial apical domain expansion, increased Notch activity and reduced neurogenesis in the zebrafish retina

Brian Clark[a], Shuang Cui[b], Joel B. Miesfeld[b], Brian A. Link[b]
[a]*Medical College of Wisconsin, Cell Biol, Neurobiol, & Anatomy, Milwaukee, WI, USA*
[b]*Milwaukee, USA*

Apico-basal cell polarity is mediated by the antagonistic functions of the apical promoting Crumbs and Par complexes and the basolateral Scribble complex, defining boundaries for junctional and asymmetric protein localization. We have examined the consequences of depletion of lethal giant larvae 1(Llgl1), a Scribble complex component, on the developing neuroepithelium of the zebrafish retina. Analyses revealed that retinal neuroepithelial cells deficient for Llgl1 maintained overall apico-basal polarity but showed expansion of the apical domain. In addition, progenitor cells showed reduced rates of neurogenesis and increased Notch reporter activity. As Notch signaling is known to maintain a proliferative state in retinal progenitors, we next sought to examine if the increased Notch signaling was a direct consequence of apical domain expansion. Apical reduction is mediated through the Shroom3-dependent apical localization of myosin II, enabling constriction of the apical actin belt. We generated a dominant negative (DN) Shroom3 transgene to expand the apical membrane in retinal neuroepithelial cells and examined consequences on both Notch signaling and retinal neurogenesis. Expression of the Shroom3DN transgene resulted in expansion of the apical domain, increased Notch signaling, and reduced neurogenesis. As apical domain size in neuroepithelia has previously been correlated with neurogenic fates, cumulatively, our data support a direct, influential role for apical domain size in selection of neurogenic progenitors or fates of their progeny.

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Program/Abstract # 259
The role of Gsx2 in the choice between neuronal versus oligodendroglial fates

Heather Chapman, Zhenglei Pei, Ronald Waclaw, Masato Nakafuku, Kenneth Campbell
Cincinnati, OH, USA

Multipotent neural progenitor cells initially give rise to neurons, however at later stages of embryogenesis they switch from neurogenesis to gliogenesis, generating predominately oligodendrocytes and astrocytes. This occurs in a specific spatial and temporal pattern, and the molecular mechanisms regulating this switch are not fully understood. The homeobox gene Gsx2 has previously been shown to be required for the specification of specific neuronal subtypes, however its role in the generation of oligodendrocytes remains unknown. A previous study showed that loss of Gsx2 leads to an upregulation of the oligodendroyte precursor cell (OPC) marker PDGF receptor, suggesting a repressive role for Gsx2 in the specification of this glial cell type. We have utilized both gain-of-function and loss-of-function approaches in order to elucidate the role of Gsx2 in the switch between neurogenesis and oligodendrogenesis within the telencephalon. In the absence of Gsx2 expression, an increase in oligodendrogenesis with a concomitant decrease in neurogenesis is observed at mid stages of embryogenesis, which subsequently leads to an increased number of Gsx2-derived OPCs within the cortex at late embryonic stages. Complementing these results, mice that over-express Gsx2 throughout the telencephalon display a significant decrease in the number of cortical OPCs at late embryonic stages. These results support the notion that high levels of Gsx2 are able to repress OPC specification, and that downregulation of Gsx2 is required for the transition from neurogenesis to oligodendrogenesis to occur normally.

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Program/Abstract # 260
The protein tyrosine phosphatase Shp2 is required for oligodendrogenesis in the telencephalon

Ronald Waclaw[a], Diana Nardini[b], Lisa Ehrman[b], Sarah Ehrman[c], Tilat Rizvi[b], Jeffrey Robbins[b], Masato Nakafuku[b]